Remarks

Claims 1, 3-5, 7, 8, 10, 12, 13, 16-21, and 24-41 were pending in the subject application. By this Amendment, claims 1, 5, 10, 16, 17, 20, and 37-40 have been amended, claims 13, 35, 36, and 41 have been cancelled, and new claims 42-48 have been added. The undersigned avers that no new matter is introduced by this Amendment. Support for the new claims and amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 1, 3-5, 7, 8, 10, 12, 16-21, 24-34, 37-40, and 42-48 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

Applicant and Applicants' representative wish to thank Examiner Long for the courtesy of the telephonic interviews conducted with the undersigned on March 5, 2009 and May 6, 2009. The undersigned wishes to clarify that, during the telephonic interview on March 5, 2009, a request was made to be notified when further examination would commence. A delay in prosecution was not requested. During the telephonic interview of May 6, 2009, the unconsidered references submitted with the Information Disclosure Statement filed on April 13, 2009 were discussed. As indicated in the Examiner's Interview Summary mailed May 12, 2009, the Examiner initially overlooked these references, yet agreed to acknowledge that all the references were considered in the next Office Action without the need for a new Information Disclosure Statement. The remarks and amendments set forth herein are consistent with the substance of the interview and are believed to address the outstanding issues as discussed during the interview.

Submitted herewith is a Request for Continued Examination (RCE) under 37 CFR §1.114 for the subject application.

By this Amendment, claims 1, 5, 10, 16, 17, 20, and 37-40 have been amended and claims 42-48 have been added. Support for the amendments to claims 1, 5, 10, and 17 can be found, for example, at page 5, lines 25-28; page 22, lines 30-31; page 23, lines 1-2; page 28, lines 1-12 (Example 4); page 29, lines 1-10; and Figure 4 of the specification as filed. Claim 16 has been amended for antecedent basis. Support for the amendments to claims 37-40 can be found, for example, at page 8, lines 13-15; and page 28, lines 16-22 of the specification as filed. Support for

new claims 42-44 can be found, for example, at pages 11-13 of the specification. Support for new claims 45-48 can be found, for example, at page 5, lines 16-24; page 28, lines 23-31; and Figures 3A-3C of the specification as filed.

Claims 1, 3-5, 7, 8, 10, 12, 13, 16-21, 24-28 and 30-36 are rejected under 35 USC §103(a) as being obvious over Hart (*Exp. Opin. Ther. Patents,* 2000, Vol. 10, pp. 199-208) in view of Ni *et al.* (U.S. Patent Application No. US2002/0151009). Applicant respectfully traverses this ground of rejection.

As an initial matter, at page 6 of the Office Action, the Examiner notes that decreased induction of interleukin-6 (IL-6) is not recited in the rejected claims. By this Amendment, Applicant has amended claims 1 and 5 to recite that "said nanoparticle induces production of less interleukin-6 in respiratory epithelium compared to a particle comprising a complex of the chitosan, or chitosan derivative, and the polynucleotide without the lipid." Applicant has also amended claims 10 and 17 to recite that the nanoparticles are administered to the respiratory epithelium and "wherein said nanoparticle induces production of less interleukin-6 compared to a particle comprising a complex of the chitosan, or chitosan derivative, and the polynucleotide without the lipid." The nanoparticles of the invention induce less IL-6, a pro-inflammatory cytokine, compared to chitosan-DNA particles without a lipid. Example 4 of the subject specification compares the effect of chitosan-lipid nanoparticles on IL-6 level, relative to chitosan and Lipofectin individually. Mice were intranasally given vector plasmid pVAX complexed with chitosan, with Lipofectin, or in chitosan-lipid nanoparticles, and IL-6 production was examined after 4 hours. Figure 4 shows a graphical representation of the results, which demonstrate that IL-6 levels were significantly reduced when using chitosan-lipid-DNA nanoparticles compared to chitosan-DNA complexes and, therefore, represent a safer gene delivery system. Applicant respectfully submits that reduction of IL-6 induction is unexpected in view of the Hart and Ni *et al.* publications.

As indicated in the Manual of Patent Examining Procedure (MPEP 716,02(a)), "a greater than expected result is an evidentiary factor pertinent to the legal conclusion of obviousness ... of the claims at issue." *In re Corkill*, 711 F.2d 1496, 226 USPQ 1005 (Fed. Cir. 1985). Evidence of a greater than expected result may also be shown by demonstrating an effect that is greater than the

sum of each of the effects taken separately (*i.e.*, "synergism"). *Merck & Co. Inc. v. Biocraft Laboratories Inc.*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), *cert. denied*, 493 U.S. 975 (1989). Evidence of unobvious or unexpected advantageous properties, such as superiority in a property the claimed compound shares with the prior art, can rebut *prima facie* obviousness. "Evidence that a compound is unexpectedly superior in one of a spectrum of common properties . . . can be enough to rebut a *prima facie* case of obviousness." No set number of examples of superiority is required. *In re Chupp*, 816 F.2d 643, 646, 2 USPQ2d 1437, 1439 (Fed. Cir. 1987).

Not only did the chitosan-lipid nanoparticles of the invention induce less IL-6, but they also exhibited greater transfection efficiency in lung epithelial cells. The cited references do not suggest that a combination of chitosan and a lipid into a single particle would be desirable or would improve transfection efficiency beyond that provided by chitosan and lipids individually. On the contrary, the inventor surprisingly found that such a combination does, in fact, achieve enhanced transfection *in vivo*. New claims 46 and 47 recite that the nanoparticle "exhibits a higher transfection efficiency compared to each of: (a) said polynucleotide alone, (b) a complex of said polynucleotide and said chitosan or chitosan derivative, and (c) a complex of said polynucleotide and said lipid." New claims 48 and 49 recite that "administering of said nanoparticle achieves higher transfection efficiency compared to administration of each of: (a) said polynucleotide alone, (b) a complex of said polynucleotide and said chitosan or chitosan derivative, and (c) a complex of said polynucleotide and said lipid."

Example 3 of the subject specification describes an experiment conducted to determine the transfection efficiency of chitosan-lipid nanoparticles in target lung epithelial cells. Groups of BALB/c mice were administered intranasally with 25μg of total pEGFP DNA (plasmid encoding green fluorescent protein (GFP)) complexed with chitosan, Lipofectin (a cationic liposome preparation), or in chitosan-lipid nanoparticles. Figure 3C shows a graphical representation of the results in which bar "1" indicates transfection with chitosan alone, bar "2" indicates transfection with Lipofectin alone, bar "3" indicates transfection with chitosan-lipid nanoparticles, and bar "4" is DNA alone. As shown by this comparative data, chitosan-lipid nanoparticles induced a 30% transfection rate in lung cells compared to the ~20% transfection rate induced by chitosan and Lipofectin alone, demonstrating that chitosan-lipid nanoparticles represent a more efficient delivery system.

The Hart publication is cited as teaching chitosan/DNA nanoparticle compositions. The Ni *et al.* publication is cited as teaching DNA/chitosan and DNA/lipid compositions. The Examiner concludes that it would have been obvious to a person of ordinary skill in the art at the time the invention was made to produce the claimed nanoparticles, and use the nanoparticles for delivery of a polynucleotide to a mammal with a reasonable expectation of success. The Examiner's rationale appears to rely on the following premise set forth in §2144.06:

> "It is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." *In re Kerkhoven*, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).

Assuming *arguendo* that chitosan and lipids were recognized in the art to be equivalents for the purpose of DNA delivery, Applicant respectfully submits that this does not render obvious a nanoparticle comprising a complex of these elements, or use of the nanoparticle to deliver a polynucleotide to respiratory epithelium. The Examiner has provided no evidence to support a reasonable expectation at the time the application was filed that the polynucleotide within the complex would be effectively expressed *in vivo*, or even *in vitro*.

The Hart publication describes nanoparticles composed of cDNA and chitosan (page 203, section 3.4) and lipopolyplex vectors composed of a combination of a lipid and a cationic polymer (page 203, section 4). However, sections 3.4 and 4 of Hart point one skilled in the art toward the utilization of proteins and peptides to increase the transfection efficiency of these respective structures (*e.g.*, transferrin ligands; peptides derived from a histone, a nucleoline, or a protamine; small viral peptides such as influenza virus haemagglutinin HA-2; apoE-3 fragment), not toward particles comprising a combination of chitosan or a chitosan derivative, a lipid, and a polynucleotide, as recited in the currently pending claims. In fact, the Hart publication indicates that in nanospheres incorporating chitosan, reporter gene expression in mice was higher and more sustained than that achieved by naked DNA and a lipid (LipofectAMINE) (page 203, section 3.4). This does not suggest that there would be any advantage to incorporating a lipid into nanoparticles composed of DNA and chitosan. In addition, the lack of *in vitro-in vivo* correlation in transfection efficiency of

nanopolyplexes relative to LipofectAMINE reported in section 3.4 of Hart does not support a reasonable expectation of success.

Not only do the cited references fail to suggest combining chitosan and a lipid in a single nanoparticle, they give no hint of the unexpected benefits conferred by their combination as demonstrated in the subject specification. Applicant respectfully submits that the claimed invention is not obvious over the cited references. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §103(a) is respectfully requested.

Claims 37-41 are rejected under 35 USC §112, second paragraph, as indefinite. By this Amendment, claims 37-40 have been amended to recite "a plurality of" polynucleotide-lipid inverted cylindrical micelles. Claim 41 has been cancelled. Support for these amendments can be found, for example, at page 8, lines 13-15; and page 28, lines 16-22 of the specification as filed. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §112, second paragraph, is respectfully requested.

Claims 1, 5, 10, 21, 25, 28, 29, 31, 37-39 and 41 are rejected under 35 USC §103(a) as obvious over Yu *et al.* (U.S. Patent Application No. US2003/0186916) in view of Vijayanathan *et al.* (*Biochemistry*, 2002, Vol. 41, pp. 14085-14094). Applicant respectfully traverses this ground of rejection.

The Examiner asserts that Yu *et al.* teach "a vector for transfecting a eukaryotic cell, comprising a nucleic acid, a nucleic acid binding polymer, a lipid-based vesicle" and that "preferred types of nucleic acid binding polymers include polymers...[such as] chitosan". Vijayanathan *et al.* is cited for teaching that non-viral delivery vehicles comprising polycationic lipids and cationic polymers condense DNA into nanoparticles. The Examiner asserts that particles comprising chitosan, or a chitosan derivative, a lipid; and a polynucleotide; nanoparticles encompassing this general formula; and the hexagonal structure intrinsic to such particles are taught by Yu *et al.* or Vijayanathan *et al*. The Examiner concludes that it would have been obvious to a person of ordinary skill in the art at the time the invention was made to produce the nanoparticles of the invention, and use them for delivery of a polynucleotide to a mammal with a reasonable expectation of success.

As indicated above, Applicant has amended claims 1 and 5 to recite that "said nanoparticle induces production of less interleukin-6 in respiratory epithelium compared to a particle comprising

a complex of the chitosan, or chitosan derivative, and the polynucleotide without the lipid." Applicant has also amended claims 10 and 17 to recite that the nanoparticles are administered to the respiratory epithelium and "wherein said nanoparticle induces production of less interleukin-6 compared to a particle comprising a complex of the chitosan, or chitosan derivative, and the polynucleotide without the lipid." The nanoparticles of the invention induce less IL-6, a pro-inflammatory cytokine, compared to chitosan alone. Applicant respectfully submits that reduction of IL-6 induction is unexpected in view of the Yu *et al.* and Vijayanathan *et al.* publications.

Furthermore, assuming *arguendo* that chitosan and lipids were recognized in the art to be equivalents for the purpose of DNA delivery, Applicant respectfully submits that this does not render obvious a nanoparticle comprising a complex of these elements, or use of the nanoparticle to deliver a polynucleotide to respiratory epithelium. The Examiner has provided no evidence to support a reasonable expectation at the time the application was filed that the polynucleotide within the complex would be effectively expressed *in vivo*, or even *in vitro*.

Applicant notes that, at page 17 and 18 of the Office Action, the Examiner indicates that:

> (1) "Since the teaching of the Vijayanathan *et al.* publication encompass nanoparticles comprising the same materials as Yu *et al.* and the instant claims, the examiner concludes the arrangement of such nanoparticles into a hexagonal lattice is a natural consequence of the chemical nature of these particles";
> (2) "it would be therefore predictably obvious to use a combination of these elements in a DNA-nanoparticle"; and
> (3) "an artisan would have expected success, because both references teach condensed DNA particles".

Applicant submits that these conclusions are not supported by the Vijayanathan *et al.* publication, which makes clear that research in the area of DNA nanoparticle formation is not so predictable, stating:

> Although significant progress has been made in the development of DNA condensation agents, the technology is complicated by a lack of understanding of the mechanism(s) of action of gene delivery vehicles and the multiple morphologies and structures of the DNA complexes with these vehicles... However, the transfection activity of the DNA-carrier complexes is highly dependent on the morphology of the condensates, indicating that the nature of the condensing agent strongly influences the properties of the condensates. A lack of clear structure-activity relationship between transfection agents and transfection efficiency makes the rationale for the

design of new delivery vehicles with high transfection activity difficult. Page 14091 of Vijayanathan *et al.*

Applicant respectfully submits that the claimed invention is not obvious over the cited references. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §103(a) is respectfully requested.

Claims 29 and 37-41 are rejected under 35 USC §103(a) as obvious over Hart (*Exp. Opin. Ther. Patents,* 2000, Vol. 10, pp. 199-208) in view of Ni *et al.* (U.S. Patent Application No. US2002/0151009) as applied to claims 1, 5, 10, 17 and 21, and further in view of Vijayanathan *et al.* (*Biochemistry*, 2002, Vol. 41, pp. 14085-14094). Applicant respectfully traverses this ground of rejection.

The Examiner asserts that Vijayanathan *et al.* teach that non-viral delivery vehicles comprising polycationic lipids and cationic polymers condense DNA into nanoparticles with columnar hexagonal liquid crystalline structures. The Examiner concludes that it would have been obvious to a person of ordinary skill in the art at the time the invention was made to produce the nanoparticles having the claimed structures and use them for delivery of a polynucleotide to a mammal with a reasonable expectation of success.

As indicated above, assuming *arguendo* that chitosan and lipids were recognized in the art to be equivalent for the purpose of DNA delivery, Applicant respectfully submits that this does not render obvious a nanoparticle comprising a complex of these elements, or use of the nanoparticle to deliver a polynucleotide to respiratory epithelium. The Examiner has provided no evidence to support a reasonable expectation at the time the application was filed that the polynucleotide within the complex would be effectively expressed *in vivo*, or even *in vitro*.

The Hart publication indicates that in nanospheres incorporating chitosan, reporter gene expression in mice was higher and more sustained than that achieved by naked DNA and a lipid (LipofectAMINE) (page 203, section 3.4). This does not suggest that there would be any advantage to incorporating a lipid into nanoparticles composed of DNA and chitosan. In addition, the lack of *in vitro-in vivo* correlation in transfection efficiency of nanopolyplexes relative to LipofectAMINE reported in section 3.4 of Hart does not support a reasonable expectation of success.

Furthermore, Applicant has amended claims 1 and 5 to recite that "said nanoparticle induces production of less interleukin-6 in respiratory epithelium compared to a particle comprising a complex of the chitosan, or chitosan derivative, and the polynucleotide without the lipid." Applicant has also amended claims 10 and 17 to recite that the nanoparticles are administered to the respiratory epithelium and "wherein said nanoparticle induces production of less interleukin-6 compared to a particle comprising a complex of the chitosan, or chitosan derivative, and the polynucleotide without the lipid." The nanoparticles of the invention induce less IL-6, a pro-inflammatory cytokine, compared to chitosan-DNA complexes lacking a lipid. Applicant respectfully submits that reduction of IL-6 induction is unexpected in view of the Hart, Ni *et al,* and Vijayanathan *et al.* publications.

Furthermore, the increase in transfection efficiency recited in new claims 45-48 is unexpected in view of the Hart, Ni *et al,* and Vijayanathan *et al.* publications.

Applicant respectfully submits that the claimed invention is not obvious over the cited references. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §103(a) is respectfully requested.

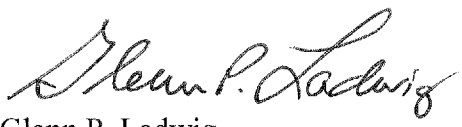
It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicant's agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicant believes that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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